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A Toxicological and Histophysiological Study of Certain New Insecticides as "Stomach Poisons" to the Honey Bee *Apis mellifera* L.

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(Conclusion)

The Effect of DDT on the Mid-Gut of the Bee

The mid-gut from twelve bees poisoned with the pure para isomer of DDT was studied. Of these, five contained the large bubble of gas described on page 48: their histological appearance was almost identical but it differed in some ways from those which contained no bubble.

The epithelial lining of mid-gut containing the gas bubble was stretched and as a result had lost its normal wavy outline especially in the mid region where the diameter of the bubble was the greatest (Pl. 1 Fig. 5). Here the epithelial layer appeared very thin. The shape of the epithelial cells varied considerably and the outlines of some were indefinite. The tips of most cells were greatly elongated, almost completely vacuolated (Pl. 1 Fig. 5-VC) and appeared to be in the process of entering free into the lumen. They may have contained digestive products as presumably did the goblet-like elements in the normal mid-gut. The part of the epithelial cells which remained in contact with the basement membrane usually contained a noticeably granular nucleus (Pl. 1 Fig. 5-GN). The cytoplasm of these cells usually stained darkly and evenly except around the nuclei where large vacuoles or lightly staining granular cytoplasm were observed. This vacuolization was much more noticeable than that described as surrounding the nuclei in a normal mid-gut. The nidi cells, possibly because of the stretched epithelial layer, seemed to have been reduced in size and, therefore, were difficult to distinguish. The only content of some nidi cells was their nuclei. The striated border was disrupted by the elongate cells and could be seen only occasionally in the hollows between the cell tips. It usually appeared as a granular mass with no apparent striations. No peritrophic membrane in the process of formation could be seen in sections cut through the area of the gas bubble. The membranes in the centre of the lumen seemed to have broken into small pieces. It seemed possible that this may have been caused either by the pressure of the bubble or by some digestive process. Small granules and larger more darkly staining elements were interspersed between the pieces of membrane in the centre of the lumen (Pl. 1 Fig. 5-PM). These were probably digestive elements and food particles (pollen) somewhat similar to those observed in the normal ventriculus. The epithelial layer of the fore and hind regions was not stretched to such an extent as that of the mid region but the wavy outline of the normal epithelium was still lacking. Secretory activity was greatly increased; and many of the resulting proliferated cells were vacuolated and appeared somewhat like the goblet-like elements of the normal mid-gut. The epithelial cells contained a number of large vacuoles especially around the nuclei, and the cytoplasm seemed to thin out, stain lightly and appear granular. The proliferation of vacuolated elements by the epithelial cells appeared greater in mid-guts containing the gas bubble. This increased proliferation may have been due either to osmotic pressure changes caused by

the gas bubble or to an increased metabolic activity of the epithelial cells. In no normal mid-gut studied was the secretory activity as great as that observed in any mid-gut from DDT-poisoned bees.

In the other seven ventriculi of DDT-poisoned bees studied the histological detail, in some ways, resembled that of a normal mid-gut and the wavy outline of the cells was quite noticeable (Pl. 1 Fig. 6). The epithelial cells at the crests of the waves had become attenuated and their cytoplasm was granular and sometimes vacuolated. Many of the tips of these cells contained the nuclei and entire cells appeared to be in the process of proliferation (Pl. 1 Fig. 6-PC). The nidi cells and the cells making up the walls of the waves were greatly vacuolated and many of their nuclei appeared granular. The striated border was still visible as lightly staining lines in the hollows between the wave-like epithelial cells (Pl. 1 Fig. 6-SB). The last formed peritrophic membrane was not in contact with the striated border in any sections studied and no new membrane could be seen in the process of formation. It appeared, therefore, that the special form of secretory activity associated with the formation of peritrophic membranes (see normal mid-gut, page 49,) had practically ceased.

No histological differences in the muscle layers were noticed.

The Effect of Acid Lead Arsenate on the Mid-gut of the Bee

The mid-gut from fourteen bees poisoned with acid lead arsenate was studied microscopically. The histological appearance of these mid-guts varied in the three different regions studied but the general appearance did not vary in different specimens. The fore and mid regions appeared more affected than the hind region (Pl. 2, compare Figs. 1 and 2, with Fig. 3). This may have been because the arsenic came in contact with these regions first.

The wavy outline of the normal epithelium was greatly modified in all regions. In the hind region most of the elongated epithelial cells on the crests of the waves were proliferated into the lumen as entire nucleated cells (Pl. 2 Fig. 3-PC). Some of the cells which were not proliferated became bent and lay over the nidi cells. The small goblet-like elements so noticeable in the normal mid-gut were almost entirely absent. The nidi cells were vacuolated and along with their nuclei had degenerated into a granular mass containing a few darkly staining areas (Pl. 2 Fig. 3-NC). The cytoplasm of the epithelial cells was granular and usually contained many vacuoles. In the fore region of the ventriculus and extending back to the mid-region, many of the epithelial cells were entirely separated from, or were in the process of separation from, the basement membrane and appeared as small groups in the lumen (Pl. 2 Figs. 1 and 2). The outline of many cells and nuclei were wholly indefinite. Similar changes in the epithelial lining of the mid-gut of the migratory locust, *Locusta migratoria*, and of larvae of the mosquito, *Aedes*, poisoned with arsenicals were found by Parfentjev (1929)—reviewed by Shephard (1939). Pilat (1935) also found that certain arsenicals caused a separation of the epithelium from the basement membrane especially near the anterior end of the mid-gut of certain Lepidopterous larvae and of nymphal migratory locusts. Both Pilat and

PLATE 2

Photomicrographs of transverse sections of the ventriculi from "dead" and from arsenic and parathion-poisoned bees. Sectioned at eight microns and stained in Heidenhain's Haematoxylin.

Fig. 1 Arsenic-poisoned bee, fore region, fixed in Bouin's. Mag. 120 x linear.
Fig. 2 Arsenic-poisoned bee, mid region, fixed in Kahle's. Mag. 110 x linear.
Fig. 3 Arsenic-poisoned bee, hind region, fixed in Kahle's. Mag. 110 x linear.
Fig. 4 Parathion-poisoned bee, mid region, fixed in Bouin's. Mag. 100 x linear.
Fig. 5 "Dead" bee, fore region, fixed in Bouin's. Mag. 150 x linear.

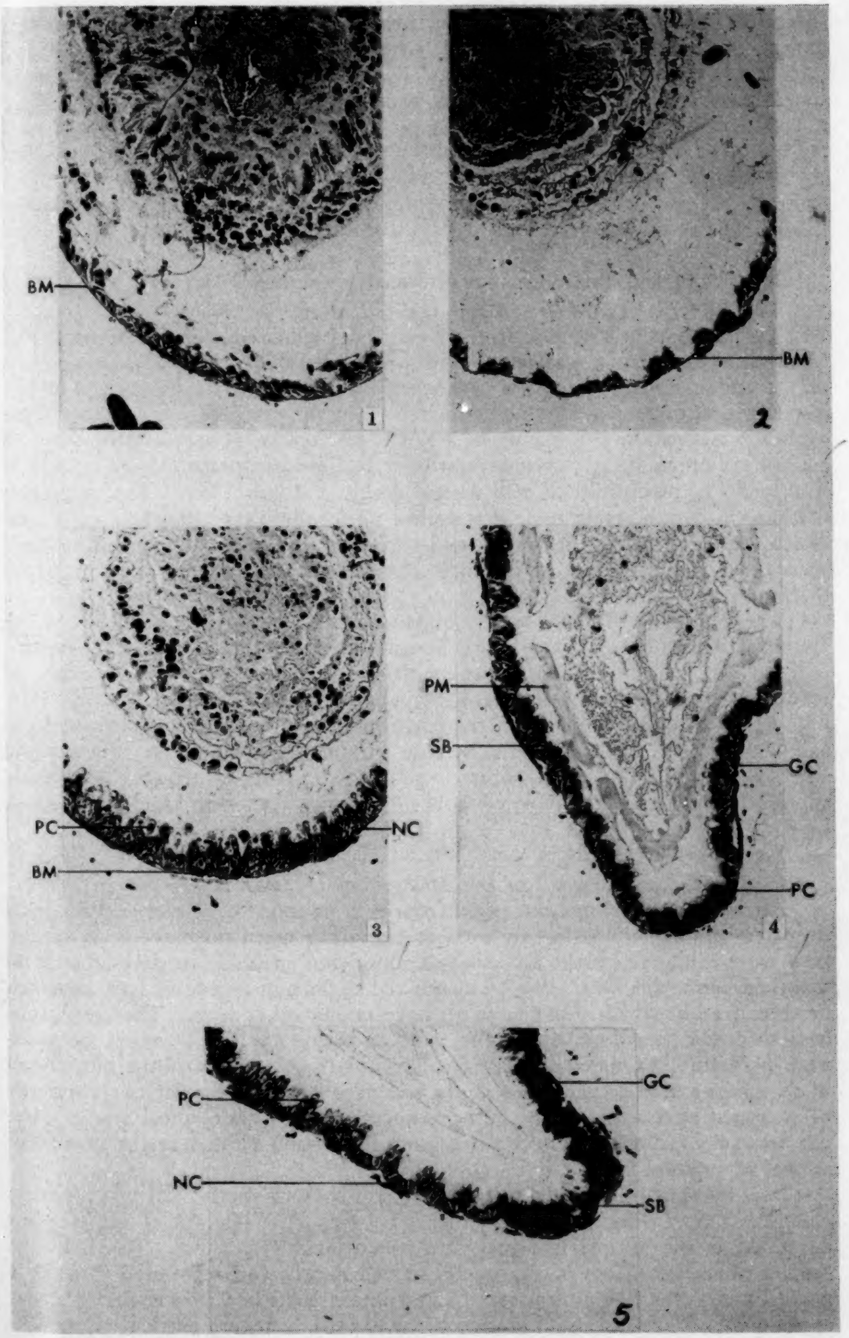

PLATE 2

Parfentjev observed that the basement membrane remained intact, a condition similar to that observed in this work when the basement membrane appeared as a darkly staining layer (Pl. 2 Figs. 1, 2 and 3-BM). The striated border appeared as a more or less granular mass in the hollows between the long epithelial cells in the hind region, but no border was seen in the fore or mid regions. No peritrophic membrane in the process of formation could be seen in any section of the mid-gut studied. However, the lumen was crowded with many concentric convoluted peritrophic membranes enclosing many darkly staining bodies which were, presumably, proliferated epithelial cells (Pl. 2 Figs. 1, 2 and 3).

No histological differences were noticed in the muscle layers.

The Effect of Parathion on the Mid-gut of the Bee

The mid-gut was studied from twenty bees poisoned with parathion. The histological appearance was only slightly abnormal. The typical wavy outline of the epithelium was apparent in all sections studied (Pl. 2 Fig. 4). Goblet-like elements of secretion were as numerous in these sections as in those from a normal epithelium (Pl. 2 Fig. 4-GC). A few epithelial cells and or parts of cells on the crests of the waves were proliferated into the lumen (Pl. 2 Fig. 4-PC). The nuclei of practically all cells stained darkly and uniformly. The cytoplasm of the cells for the most part, also stained uniformly although a few cells contained vacuoles. As in the normal mid-gut, the nuclei were surrounded by clear spaces, but in only a few of the nidi cells did these spaces appear to be larger than normal.

The striated border was usually quite noticeable following the outline of the epithelial cells and the definite lightly staining lines could be seen as in the normal mid-gut (Pl. 2 Fig. 4-SB). The peritrophic membrane in the process of formation appeared as a wide darkly staining band in all sections studied (Pl. 2 Fig. 4-PM). This differed from the peritrophic membrane in a normal mid-gut and may be the result of the action of parathion on the secretion from the striated border. The peritrophic membranes in the centre of the lumen appeared as the usual convoluted concentric rings enclosing small granules of food.

No histological changes were noticed in the muscle layers.

The Effect of "Death" on the Mid-gut of the Bee

Although the mid-gut was taken from each poisoned bee *before* actual death occurred (death defined in this work as occurring when no movements of any kind were exhibited by the bee), it was considered advisable to study that from a normal bee which was "dead", i.e., showed slight movements of legs, abdomen or antennae but which was unable to move in any other way. Therefore, four bees that had been kept under the same conditions as the poisoned bees, see page 44, were decapitated and left on the laboratory bench until the movements of their legs and abdomens, (see above and page 43) were similar to movements of poisoned bees immediately before evisceration for histological study. The gut was removed then from the decapitated bees and treated in the same way as that of poisond bees.

The mid-gut of each decapitated bee still showed the typical wavy outline of epithelium of a normal mid-intestine (Pl. 2 Fig. 5). The goblet-like elements described in the normal mid-gut were still present (Pl. 2 Fig. 2-GC), but in smaller numbers. Most of the nuclei stained darkly and resembled those of a normal mid-gut. The cytoplasm of the epithelial cells had a somewhat granular appearance. Vacuoles could be seen in many cells, but were particularly noticeable in the nidi cells (Pl. 2 Fig. 5-NC). Entire nucleated epithelial cells and

or parts of cells appeared to be in the process of proliferation (Pl. 2 Fig. 5-PC), but this activity was no greater than in a normal mid-gut. The striated border was still present as a faintly staining granular mass (Pl. 2 Fig. 5-SB). The striations were not so noticeable as in a normal mid-gut but a peritrophic membrane could be seen near the tips of the epithelial cells as though it were in the process of formation. The lumen contained a few concentric peritrophic membranes.

The general appearance of the mid-gut from a normal "dead" bee (decapitated three hours before evisceration) was very much like that of a normal bee. It, therefore, seems probable that any histological abnormalities observed in the mid-gut of poisoned bees were due to some direct or indirect action of the insecticides.

Discussion

Feeding technique, age of test insects, size of cage, numbers of insects per cage and the nutrition of the test insect may all have an effect on the mortality figures in laboratory insecticidal tests (Morrison, 1943). In the present investigation, the test bees used were of approximately the same age and were taken from the same population. A conditioning time of twenty-four hours at the laboratory and incubator temperatures was used in an attempt to ensure that all bees tested would be in the same physiological state. (Later it was found that this time was unnecessarily long). The individual feeding technique was used to give an absolute control over the amount of insecticide which each bee received, and all tests were carried out at 30°C, a temperature found by the Apiculture Department, O.A.C., according to Professor G. F. Townsend, to be the biological optimum for honey bees.

Since check bees exhibited no ill effects when pollen was used as a suspending agent for DDT and when 95% ethyl alcohol was used to make the parathion miscible with sugar syrup, it was thought that a reliable comparison of the effect of these insecticides on the mid-gut of the honey bee could be made even though these materials were used as suspending agents.

Mortality counts in all tests were taken twelve hours after the poison solutions had been removed and, therefore, slow-acting insecticides such as arsenic and DDT would have a high median lethal dose in this work. Acid lead arsenate was found to be less toxic (L.D. 50=204 micrograms)[3] than the 50% DDT (L.D. 50=131.8 micrograms) in this experiment probably because of the longer time required for the arsenic to kill. Parathion, however, was the fastest acting and the most toxic insecticide tested (L.D. 50=0.095 micrograms). This value was in agreement with that found by Eckert (1948) of 0.07 micrograms and by Häfliger (1949) of 0.1 to 0.09 micrograms per bee, even though the experimental as well as the physiological conditions of the test insects were different in the three separate investigations; i.e. Eckert, Häfliger, and this work. The L.D. 50 for the 50% DDT was approximately twice that for the pure para isomer of DDT. (L.D. 50=54.9 micrograms). Since the Wettable Powder contained only 50% of a technical grade of DDT this result would be expected: for it may reasonably be supposed that 50% of technical grade DDT would contain less than 50% pure pure para isomer DDT.

Little has been written concerning the standardizing of techniques for histological studies. Throughout this investigation attempts were made to keep all histological procedures standard. All mid-guts to be compared histologically were subjected to the same fixation, dehydration and embedding times, see page 44. It seemed reasonable to assume that any histological differences between

[3]All median lethal doses (L.D. 50) expressed as micrograms per bee.

a poisoned and a normal mid-gut treated with the same fixative, would be due either directly or indirectly (e.g. artifacts, see page 44) to the poison. For this reason and because tissue fixed in Mann's solution had a tendency to crack and crumble, all comparative histological studies were made with tissues fixed in Bouin's or Kahle's solution—fixation by these two solutions being similar.

A normal resting epithelial cell was difficult to identify because of the variation in secretory activity from cell to cell and from region to region in the same gut (Snodgrass, 1925). Sections were therefore cut from three different regions. In any one observation, sections from the same region of both a normal and a poisoned mid-gut were put on the same slide and stained at the same time, so as to allow true comparison of the histological structure and nullify any differences that might have resulted from slight variations in staining technique.

The difficulties of making comparisons in morbid histology are twofold. Firstly, there is the possibility of confusing ordinary post-mortem changes with those due to the poisons; and secondly, there is the question, how long after the administration of a poison should microscope observations be made? These difficulties are surmounted by students of the vertebrates and by medical and veterinary scientists. There seemed no reason for thinking them insurmountable in insect studies. Actually two precautions were taken: (1) mid-guts were always taken from poisoned bees exhibiting the same symptoms, i.e. slight movement of legs, abdomen or antennae (see page 43). This was considered advisable because of the different times required by the insecticides to kill; (2) mid-guts were removed from bees which had been decapitated and left till they exhibited the same external physiological condition as the poisoned bees. Guts from these bees acted as a check on post-mortem changes.

The great amount of vacuolization and of apparent secretory activity in the mid-gut cells of the twelve DDT-poisoned bees studied, suggested that some metabolic disturbance was caused by the insecticide. Merrill et al (1946) working with the American cockroach *Periplaneta americana*, Ludwig (1946) with the Japanese beetle *Popillia japonica* and Barron (quoted by Metcalf, 1943) with the yellow meal worm adult *Tenebrio molitor*, all suggested that the ultimate cause of death in DDT-poisoned insects was metabolic exhaustion caused by hyperactivity and the prolonged period of prostration before death occurred. The hyperactivity observed in DDT-poisoned bees suggested that this insecticide was acting as a neuromuscular poison (cf. Bodenstein, 1946, Welsh and Gordon, 1947). It is, perhaps, possible that DDT administered as a stomach poison is absorbed by the epithelial cells of the mid-gut and then affects the nerves of the stomatogastric nervous system, for Bodenstein (1946) found that DDT could affect the peripheral nervous system without necessarily affecting the central nervous system. The presence of a large gas bubble in the mid-gut of some (five) DDT-poisoned bees suggested that the insecticide might have affected the frontal ganglion of the stomatogastric nervous system for this ganglion controls the centre of deglutition (Wigglesworth, 1942); and it is tentatively suggested that the DDT may affect the ganglion and result in an abnormal swallowing activity which would cause air to enter the mid-gut. In addition, the microscopical appearance of a mid-gut from a DDT-poisoned bee suggested a marked increase in metabolic activity. It seemed possible from a study of the actions of DDT-poisoned bees that hyperactivity might indeed be causing such a drain on the food reserves of the insect that death resulted from autolysis and metabolic exhaustion.

The microscopical appearance of all mid-guts from bees poisoned with acid

lead arsenate was found to be similar. Cellular and nuclear degeneration was the characteristic feature. The fore and mid regions were most affected (see page 49) possibly because the poison came in contact with them first, but the nidi cells of the hind regions were almost complete vacuoles, possibly because they had become worn out replacing epithelial cells.

It seems that the effectiveness of acid lead arsenate as a stomach poison is related to its digestibility or solubility in the digestive tract (Shephard, 1939). Its solubility in the digestive fluid of the ventriculus of the honey bee was found by Tietz (1924) to be a little over one and three quarter times its solubility in distilled water; moreover the buffering effect of certain solutions in the ventriculus of the honey bee has little effect on the toxicity of arsenicals (Hoskins and Harrison, 1934). Death of the epithelial cells in mid-guts from arsenic-poisoned insects was thought by Voegtlin, Dyer and Leonard (1925) to be caused by asphyxiation: arsenic inhibits the uptake of oxygen in tissues by interfering with the normal equilibrium between oxidized and reduced glutathione because of its affinity for the SH(cysteine) group of glutathione. However, evidence of this was lacking in this work.

A histological study of the mid-gut from twenty parathion-poisoned bees indicated that the physiological activity in this region was not noticeably affected. A parathion-poisoned bee showed an increase in activity almost immediately after feeding and this activity was continued until death occurred. The speed of the toxic action, the circus movements of the insect and the continuous tongue movements which usually ended in regurgitation, all indicated that the brain and the nerves close to the alimentary tract were affected. It is tentatively suggested that the frontal ganglion may be affected by this insecticide because regurgitation occurs with most poisoned bees. Mazur and Bodansky (1946) and Chadwick and Hill (1947) stated that organic phosphates (parathion) irreversibly inhibit the action of cholinesterase in mammals and in insects. As a result of this inactivation of cholinesterase, continuous impulses are conveyed across the synapses concerned. This action of parathion may explain the continuous tongue and circus movements. The speed of toxic action perhaps indicates a rapid absorption through the epithelial cells of the mid-gut or it may indicate that the insecticide acts immediately as a contact poison on the cuticular lining of the fore-gut.

While it is generally recognized that we have no certain knowledge of the exact mode of action of many insecticides, the theories suggested by various investigators do stimulate further research (Martin, 1948). From a general review of the literature and from observations made in this investigation, it seems likely that DDT, arsenic and parathion each affect the honey bee in a different manner. Parathion and DDT, although they both affect the neuromuscular system, (hyperactivity of poisoned bees), appear to do so, not only at a different speed, but in a different way, because regurgitation of the poison was a characteristic common to most parathion-poisoned bees but it was not noticed in any DDT-poisoned bees. Moreover, the normal histological appearance of the mid-guts from parathion-poisoned bees, contrasted with the increased secretory activity of the epithelium and the presence of a gas bubble in those from DDT-poisoned bees also suggested that the two insecticides were acting in a different manner. It may be that histophysiological changes caused by DDT in the mid-gut of a honey bee are not the result of any direct action of the insecticide on the epithelial cells but are rather an indirect effect (overactivity leading to metabolic exhaustion) caused by the action of DDT on the neuromuscular system of the bee. Parathion, however, appears to have neither

a direct nor an indirect effect on the mid-gut. It is tentatively suggested that both these insecticides may enter the body of the insect through the cuticular lining of the fore gut and may affect the frontal ganglion of the stomatogastric system. Arsenic, however, appears to have a direct action on the epithelial cells of the mid-gut as seen by microscopic examinations because no symptoms of neuromuscular poisoning were seen in arsenic-poisoned bees. Because the honey bee is very dependent on a continuous supply of food for life, it is possible that if the midgut epithelium were severely damaged (as it apparently is by arsenic) the bee's death might well be due partly to acute starvation.

Summary

1. The object of this work was to determine the toxicological effect of acid lead arsenate, DDT (Niatox 50% DDT and pure para isomer of DDT) and parathion on the honey bee and to observe any histological or physiological differences from normal in mid-guts from bees poisoned with these insecticides.
2. All bees used in the toxicological tests were subjected to a 24 hour conditioning time under controlled temperatures. Each bee tested was kept in a separate cage and an individual feeding technique was used to give a measured dose of insecticide to each bee. Tests were run with 2, 5 and 24 hour conditioning times to determine if a 24 hour time was necessary. Analysis of the data indicated that it was not.
3. The median lethal dose for each insecticide was found by the dosage probit method. Both DDT and arsenic are slow acting insecticides, (1-2 days) and since mortality counts were taken 12 hours after feeding the insecticides, it is natural that the L.D. 50 for these insecticides should be high in this work. Parathion was found to be a very fast-acting and toxic insecticide to honey bees.
4. The hyperactivity of DDT-poisoned bees and the hyperactivity and circus movements of parathion-poisoned bees indicated that both insecticides were acting as nerve poisons. No characteristic movements were noticed with arsenic-poisoned bees.
5. Distinct macroscopic changes were noticed in the ventriculi of some DDT and arsenic-poisoned bees: a large transparent gas bubble was seen in the ventriculi of about 50% of the DDT-poisoned bees while a mid-gut from an arsenic-poisoned bee was characterized, in 90% of the cases, by a greyish plug-like mass at or near the hind region of the gut. The mid-gut from a parathion-poisoned bee showed no abnormality. It seems possible that these observations could be used as a means of identifying insecticides causing death to honey bees in the field.
6. No apparent histological changes were noticed in the mid-gut from a parathion-poisoned bee.
7. Distinct histological differences from the normal structure were seen in mid-guts from DDT-poisoned bees. The epithelial lining was stretched (gas bubble) and increased secretory activity of the epithelial cells appeared to be characteristic features.
8. Vacuolization, defoliation and degeneration of the epithelial cells were characteristic of a mid-gut from an arsenic-poisoned bee.
9. The possible mode of action of these three insecticides are discussed.

Acknowledgments

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References

Baker, J. R. (1945). Cytological Technique (2nd ed.). London: Methuen and Company Limited.

Bliss, C. I. (1935). The comparison of dosage-mortality data. *Ann. appl. Biol., 22:* 307-33.

Bodenstein, D. (1946). Investigation of the locus of action of DDT in flies *Drosophila virilis. Biol. Bull. 90:* 148-157.

Butt, F. H. (1934). The Origin of the Peritrophic Membrane in *Sciara* and the Honey Bee. *Psyche, 41:* 51-56.

*Chadwick, L. and Hill, D. (1947). *J. Neurophysiol., 10:* 235.

Eckert, J. E. (1948). The relations of agricultural chemicals to bee-keeping. In report of the State Apiarist for 1948, pp. 64-69. State of Iowa.

Eltringham, H. (1930). Histological and Illustrative Methods for Entomologists. Oxford: Clarendon Press.

Finney, D. J. (1947). Probit Analysis, London: Cambridge University Press.

Häfliger, E. (1949). Comparative toxicity of various insecticides to the honey bee. *J. econ. Ent. 42:* 523-31.

Hartzell, A. (1945). Histological effects of certain sprays and activators on the nerves and muscles of the house fly *Musca domestica. Contribs. Boyce Thompson Inst. 13:* 443-54.

Hertig, M. (1923). The normal and pathological histology of the ventriculus of the honey bee with special reference to infection with *Nosema apis. J. Parasit., 9:* 109-140.

Hoskins, W. M. and Harrison, A. S. (1934). The buffering power of the contents of the ventriculus of the honey bee and its effect upon the toxicity of arsenic. *J. econ. Ent., 27:* 924-42.

Kennedy, C. H. (1932). Methods for the study of the Internal Anatomy of Insects. Ohio State University, Columbus, Ohio.

*Ludwig, D. (1946). The effect of DDT on the metabolism of the Japanese beetle *Popilla Japonica,* Newman. *Ann. ent. Soc. Amer., 39:* 496.

Martin, H. (1948). Some biochemical aspects of insecticide research. *Research 1:* 640-646.

*Mazur, A. and Bolandsky, O. (1946). *J. Biol. Chem., 163:* 261.

*Merrill et al. (1946). *J. Cellular Comp. Physiol., 28:* 465.

Metcalf, R. L. (1948). The mode of action of organic insecticides. National Research Council, Washington.

Morrison, F. O. (1943). The standardizing of a laboratory method for comparing the toxicity of contact insecticides. *Can. J. Research. Sec. C. 21:* 35-59.

Musgrave, A. J. (1937). The histology of the male and female reproductive organs of *Ephestia kühniella* Zeller (Lepidoptera). *Proc. Zool. Soc. Lond. Series B,* 337-364.

Musgrave, A. J. (1946). Tests on clothing impregnated with DDT as an anti-louse measure. *Bull. ent. Res., 37:* 43-56.

*Parfentjev, J. A. (1929). Researches in insect toxicology. *Trans. IV Internat. ent. Congr.,* (1928) pp. 857-64.

Pavlovsky, E. N. and Zarin, E. J. (1922). On the structure of the alimentary canal and its ferments in the bee *Apis mellifera L. Quart, J. Micr. Sci., 66:* 509-56.

Pilat, M. (1935). Histological researches into the action of insecticides on the intestinal tube of insects. *Bull. ent. Res., 26:* 165-80.

*Richards, A. G. and Cutkomp, L. K. (1945). Neuropathology in Insects. *J.N.Y. ent. Soc. 53:* 313.

Rider, F. R. (1939). An Introduction to Modern Statistical Methods, New York: John Wiley and Sons, Inc.

Shephard, H. H. (1939). The Chemistry and Toxicology of Insecticides. Minneapolis: Burgess Publishing Co.

Snodgrass, R. E. (1925). Anatomy and Physiology of the Honey Bee. New York: McGraw-Hill Book Co., Inc.

Tietz, H. M. (1924). The solubility of arsenate of lead in the digestive fluids of the honey bee. *J. econ. Ent., 17:* 471-77.

Tobias, J. and Kolros, J. (1946). *Biol. Bull., 91:* 247.

*Voegtlin, C., Dyer, H. A. and Leonard, C. S. (1925). On the specificity of the so-called arsenic receptors in the higher animals. *J. Pharm. and Exp. Therap., 25:* 297-307.

Welsh and Gordon (1947). The mode of action of certain insecticides on the Arthropod nerave axon. *J. Cellular Comp. Physiol., 30:* 147-169.

White, G. F. (1918). A note on the muscular coat of the ventriculus of the honey bee *Apis mellifera. Proc. ent. Soc. Wash., 20:* 152-154.

Wigglesworth, V. B. (1947). The Principles of Insect Physiology (2nd ed.). London: Methuen and Company Limited.

*Witt, P. (1947). Z. *Naturforsch, 2b:* 361.

*Not seen in the original.

Trends in Research in the Division of Entomology[1]

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The research program of the Division of Entomology is steadily evolving as surely as are the insects themselves. The broad outlines of today's program have been shaped through the years by central administrative policies, by the nature and significance of the problems encountered, and by the pressure of public demand; the detail has been affected primarily by the availability of trained staff and research facilities, by the quality of local leadership, and by pertinent developments in sister sciences. All of these are continuing influences, which, together with important recent developments and some weighty unpredictables, will determine the nature and scope of tomorrow's program. But certain trends in research seem clear. The subject is discussed under three main headings, with a final summarizing statement.

Trends in Relation to Administrative Policy

The Division of Entomology exists because there is a publicly recognized need for entomological services in Canada. Its program is, of necessity, built around this need. Hence, the planning *of* research remains largely a responsibility of laboratory officers, but the planning *for* research and the development of an over-all program become increasingly administrative in character as these responsibilities pass from the laboratory to the higher Divisional levels. In discharging these duties, administrators invariably develop policies concerning establishments, functions, responsibilities, and procedures. Rightly or wrongly, these policies eventually exert a profound effect on the broad outlines of the research program. This is evident today and its significance is discussed in the ensuing paragraphs.

Types of Research

For convenience in discussion, the term *research* is used in the broad sense and includes all levels of experimentation and investigation. Consequently, reference is made to simple field tests, to precise field and laboratory work, and to fundamental research.

The British North America Act (1867) confers upon the Government of Canada a responsibility for conducting researches appropriate to the needs of the country. The entomological needs are obvious. Essentially they are the protection of individuals, industry, and trade from the ravages of insects. Therefore *practical protection* is the ultimate objective. This must be kept clearly in mind at all times if the Division is to discharge its major responsibility.

Prevailing circumstances affect the choice of method that may lead to practical protection from a given insect pest. Not infrequently expediency dictates the course of action. This was particularly true in the early years of the Division, when the ratio of problems to investigators was so high that only the most urgent situations received attention and empirical methods such as those used in short-term field tests frequently were the only feasible approach. Innumerable distressing situations were alleviated by this means. Even today, with vastly increased staffs and facilities, this method—greatly improved through increased knowledge and better techniques—is often employed, and its inclusion in programs of the immediate future is inevitable. Admittedly, the empirical approach usually relieves rather than cures; but we are in no position as yet to

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scorn or discard the practical palliative. Nevertheless, it is at best a low-grade type of research and will steadily decrease in relative importance in future Divisional programs.

But all field research is not empirical. Too frequently non-entomologists fail to appreciate this fact. Through personal experience many of them have come to associate research (of the better type) with the indoor laboratory and to feel that the two are inseparable. However, hypotheses, precise techniques, controlled factors, and critical mathematical and biological interpretations can be employed outdoors—or in other practical situations such as in mills, warehouses, stables, and the holds of ships—in investigations that will yield reproducible results. Perhaps precise field research is the most difficult type of entomological investigation to be undertaken; it must, therefore, be employed with enough discretion to prevent waste of effort and funds, and with sufficient boldness and determination to exploit its virtues. This type of research is already well launched and is assured of an increasingly important place in the future program of the Division.

Until recent years, nearly all economic entomologists in the Division were field investigators. Indeed, prior to World War II there were few entomological research laboratories worthy of the name. That has been and is being changed; and if developments now in course are carried to completion, Canada will have laboratory facilities second to none. This development has been too long delayed in our science; and the laboratory entomologist can learn much from the chemist, the plant pathologist, and the microbiologist, who from the beginning have emphasized the laboratory approach to agricultural problems. Precise laboratory research is definitely on the increase and will constitute a major advance in the next decade of Canadian entomology.

The question is frequently asked, "Are officers in the Division of Entomology permitted to undertake fundamental research?" A matter of definition is involved here, but if *fundamental* be interpreted broadly the answer is "yes". To illustrate, research workers are definitely encouraged to work at a still more basic level than that currently in course if in their opinion such will contribute directly or indirectly to the solution of pest problems. This policy should provide sufficient scope and challenge to satisfy any ambitious scientist. However, academicians often prefer to define fundamental research as a quest for truth without regard for immediate application or even for any ultimate specific utility, such investigation being directed solely to the extension of knowledge of the general principles governing natural or other phenomena. This definition rules out any utilitarian purpose and, if interpreted narrowly, places fundamental research beyond the responsibility of the Division. However, the carefully planned, precise investigations referred to previously could accurately be dignified as *basic applied research*, and this compromise will probably suffice for all practical purposes. Within these limitations, *fundamental* or *basic* research is certain to increase materially in the years immediately ahead. This development is the surest means of raising the general level of the whole research program. Fortunately, the trend toward research of an increasingly fundamental character is strongly under way at present and never was Canada in so favourable a position to promote this phase of the program. There are greater numbers of specialists available, and better research facilities at hand; more important still, the total staff is becoming large enough to absorb the shock of new public demands without wholly withdrawing support from long-term basic investigations. This protection is essential if fundamental work is to thrive.

Organization and Program Planning

Other significant trends in research development include the team approach to complex problems, the construction of composite-type Science Service laboratories, the closer integration of divisional programs, the consolidation of administrative services, increased emphasis on research direction, and closer co-operation with other research institutions in Canada.

The "general practitioner" still is and always will be a key figure in applied entomology, but the relative importance of the research specialist is increasing. The logical extension of this development is the establishment of teams of specialist research workers wherever knotty basic problems exist. Since an insect is a physico-chemical mechanism, it is not surprising that the services of a meteorologist, a chemist, or a physicist are helpful in explaining certain entomological phenomena; and where insects are intimately associated with the spread of disease or where hosts exhibit immunity or resistance to insect attack, the team profits from the addition of a pathologist, a physiologist, a geneticist, or all of these.

Obviously, if such specialists are to work together effectively they must be housed together. Consequently, there has been a decided shift in recent years toward the establishment of a composite type of Science Service laboratory in preference to those that serve but a single division. This does not mean that laboratories specifically for entomology will not be built, or that purely entomological research will not be undertaken in composite laboratories. It does imply, however, that as time goes on an increasingly greater fraction of the total entomological effort will be conducted in co-operation with officers trained in other scientific disciplines. This appears to hold two important implications for the future: first, co-ordination of research programs will increase until a single highly integrated program is evolved for all related agricultural sciences; second, the development of a consolidated administration at each laboratory may eventually lead to a single consolidated administration for all divisions in the Service. These developments would call for changes in the present organization the nature and extent of which are not fully apparent as yet.

If such developments mature it is safe to predict that research direction of the whole Science Service program will receive considerably greater attention than has been possible in the past. The greatest weakness today is that those directing research are too often so overburdened with routine duties that critical planning is relegated to the status of a spare-time occupation. Care must always be taken to preserve the essential freedoms of the scientist, but efficiency, system, standards of requirements, and good organization are not at variance with these.

Obviously, the above developments do not stand alone, but are significantly related to concurrent developments in Canadian colleges and universities, from which the main supply of professional officers must come. Closer co-operation in research activities is desirable, therefore, wherever mutual advantage will accrue. The same principle should be extended so far as it is practicable to other Canadian research institutions and even internationally.

Trends in Project Development

The present program of the Division of Entomology forms a tremendous mosaic of interrelated lines of investigation. However, the broad approaches remain unaltered from one period to the next. Hence we shall go on indefinitely toward identification of pest species, prediction of impending outbreaks, and development of practical control measures. But changes do occur both in degree of emphasis and in specific lines of endeavour. The more significant of

these are discussed briefly under separate headings, but with the full realization that they are not mutually exclusive.

Throughout the discussion the term *project* is used in the generic rather than in the restricted sense.

Insect Biology

The most important trend today is the increased emphasis on the biology of insects. This must be encouraged, for true progress in insect control depends more upon this basic knowledge than upon any other factor. Greatly intensified studies on life-history, including diapause, and/or behaviour patterns are already under way in many projects, such as those associated with the spruce budworm, several species of bark beetle, wireworms, the wheat stem sawfly, the green peach aphid, warble flies, mosquitoes, black flies, bees that trip alfalfa flowers, orchard-inhabiting mites, spider beetles, and host-parasite relationships. This trend is evident in nearly every section of the Division and holds encouragement for the future.

Systematic Entomology

Accurate specific identification is a prime requirement of all entomological work. This can be accomplished only if adequately representative collections and other data are available and if competent staff have the opportunity for intensive revisional work. Unfortunately, the Systematic Entomology Unit never has been large enough to serve completely the needs of the Division. This discrepancy has been emphasized in recent years with the tremendous expansion of field projects, and taxonomy specialists have been obliged to spend too much time on routine identifications. Nevertheless, developments of real consequence have been made. During the period 1945-50 the total staff of this unit has grown from 11 to 36 and the professional staff from 7 to 18. Many more are needed, and every effort must be made to continue the trend toward the appointment of specialists in those orders and families for which the need is most urgent.

A beginning has been made in the placement of taxonomists at field laboratories where they can work jointly with economic entomologists in systematic regional collecting; preparation of practical field manuals; intensive research on local taxonomic problems, especially those pertaining to immature stages; and the screening of material to be sent to Ottawa for identification. This venture is still on a trial basis, but if it be successful some expansion may be expected.

Excellent results have been obtained from the Northern Insect Survey, begun in 1947; and systematic large-scale collecting in other parts of Canada would be the logical extension of this activity.

Quantitative Population Studies

Reliable methods for measuring the abundance of insects in the field are prerequisite to forecasting of outbreaks and appraisal of control measures. Noteworthy advances have been made in recent years both in the refinement of sampling techniques and in their application to practical problems. Good illustrations may be drawn from forest insects, soil-inhabiting pests, orchard insects, and blood-sucking flies. The significance of such work has long been recognized and the present trend assures increased attention in the future.

The factors affecting insect abundance have also been under closer scrutiny in recent years. The study of physical factors is on the ascendency, especially the micrometeorology of insect habitats. This places the emphasis on the perfecting of techniques for measuring physical factors at the exact spot where the insect exists, be it in a silken hibernaculum, a tunnel in the soil, or a gallery under bark. Of the biotic factors involved, the trend is toward more careful consideration of nutritional requirements and mortality from insect diseases.

Diseases of Insects

During the past four years the study of insect diseases has moved from a position of relative insignificance to one of major importance in the Division. It is certain to continue as a dominant activity in the future. Important current objectives include the accurate assessment of the effect of naturally occurring diseases on insect populations and the practicability of utilizing artificially induced epidemics as control agents. Such an effort requires the support of both intensive and extensive fundamental work. Fortunately, the provision of specialist staff and essential facilities is already well advanced. Undoubtedly, many of the investigations performed in this field during the ensuing decade will be among the most fundamental conducted in the Division.

Medical Entomology

Formal recognition of the importance of this field of work was made in 1947 with the establishment of the Household and Medical Entomology Unit. The research program has centred around the biology and control of biting flies; and the medical and agricultural significance of the species encountered is rapidly being revealed. Undoubtedly, the work of this unit will continue as a vigorous line of endeavour in the immediate future.

Insect Vectors of Disease Organisms

The role of insects in the transmission of plant and animal disease organisms is a field of investigation in which new problems are appearing with disturbing frequency. Experience has shown, moreover, that such problems invariably are complex and require careful work by appropriate teams of specialists to achieve even modest success. Until a better knowledge of suitable procedures and techniques is acquired, the wiser course for the future appears to lie in undertaking only the most pressing of such problems, attacking these with full force, and leaving all others strictly alone. The empirical approach is usually unproductive in this type of problem, and work should be initiated only where qualified staff and adequate laboratory facilities are available. Indeed, the consolidation of the basic aspects of this work at one or two national centres probably would prove more rewarding than further scattering of the available research resources.

Plant Parasitic Nematodes

The history of nematode problems in older agricultural countries suggests strongly that the infestations revealed in Canada in recent years are but a foretaste of things to come. There is a definite need for further research in this field, especially on the taxonomic positions of forms found in Canada and on their biologies and controls. Lack of trained nematologists is the principal factor limiting progress at present.

Beneficial Insects

Interest has heightened in recent years in the use of various species of insects for the control of weeds and in the utilization of honey bees and wild bees in the improvement of seed-setting in clovers and alfalfas. Preliminary projects in these lines have just been initiated in Canada. If these are successful, further work may be expected.

The importation into Canada—usually through the Commonwealth Bureau of Biological Control—of parasites and predators of pest species and the transfer of parasitic forms from one part of Canada to another are activities of decidedly longer standing. The greatest present need in this work appears to be for more precise information concerning the factors and principles that underlie optimum host-parasite relationships. The present trend, therefore, is toward fewer

projects, one or two at least being on a much more comprehensive scale than formerly, and toward adequate provision for appraising the effectiveness of liberations if these be made.

Insecticides

A new era lies directly ahead in the chemical control of insects. The trial and error stage is passing and the period of a planned approach is dawning.

Early in 1951, the Science Service Laboratory at London, Ont., will be opened, specifically for fundamental work. The precise directions in which such work will be cast has not been decided as yet, but the following are among the most pressing needs: (1) clarification of the factors responsible for insect resistance to insecticides; (2) proper understanding of systemic insecticides; (3) reliable information on the effect of soil treatments upon the temporary or permanent productivity of the land; (4) development of potent, highly selective insecticides with low toxicity to mammals and plants; and (5) elucidation of the basic principles underlying the interrelationships of chemical structure, formulation, and toxicity. Useful information on one or more of these important phases may be anticipated in the years immediately ahead.

An excellent beginning has been made in the improvement of machinery for the application of insecticides, the development of the orchard concentrate sprayer being particularly noteworthy. However, this whole question requires further consideration with a view to its reestablishment on an adequate Divisional, Service, or Departmental basis.

Finally, there is an urgent need for a qualified toxicologist at each of the major field laboratories serving agricultural entomology. Such an officer, by virtue of his special training, would serve as local adviser in the planning and conducting of chemical control projects and would act as local liaison specialist with the London laboratory. This or an alternative development is required if the Division is speedily to improve the quality of its chemical control investigations.

Special Techniques

With increasing emphasis on precise research both in the field and in the laboratory, entomologists will be obliged to give high priority to the development of techniques and methods appropriate to their needs. Not the least important of these will be improvement in methods of laboratory rearing of test insects, including in some instances the production of physiologically uniform strains.

Other Projects

The topics enumerated above are illustrative rather than fully descriptive of trends in relation to current needs and recent developments. Many other activities will be carried into the future. Among those most likely to demand increased attention are: plant breeding for insect-resistant varieties; improved insecticides for use on or in animals; and comprehensive ecological studies with special reference to improved management practices for the forest, the orchard, the grain farm, and the range.

Some Unpredictables

History reveals that trends in research may be profoundly influenced by a number of factors that cannot be evaluated properly in advance of their occurrence.

The present international situation is a case in point. During the past year Canada has shifted from a wholly peacetime economy to a semi-wartime basis. This may delay the completion of laboratories that are now in the blueprint stage, and so indirectly affect the research program. If another world-wide

conflict should ensue, important repercussions would follow as a result of staff changes, new agricultural and forestry priorities, new storage and transportation problems, and special military requirements.

Changes in agricultural or forestry practices, if widely adopted, affect the associated insects. This has been demonstrated in numerous instances in the past. Hence, the putting under irrigation of an additional half million acres of farm land in southern Alberta will undoubtedly affect the entomological problems of that area. Similarly, the present regular and widespread use of chemicals for the control of weeds in grain fields limits the variety of food available to plant-feeding insects and may even change the chemical composition of the crop plants themselves to a degree that will attract insect species not hitherto of economic status. Comparable possibilities exist in the forest, especially with the prospect of expanded reforestation and the general adoption of new standards in cutting and logging practices.

Applied entomologists have long profited from advances in other sciences. These developments materially affect trends in entomology because new basic information or new techniques invariably open up new fields for exploration. Research tools that have significantly influenced recent trends in entomological research include the electron microscope, radioactive isotopes, paper chromatography, serological procedures, and even the punch-card system of recording and sorting data. No one can predict what new techniques will appear next and what their influence will be.

Another rather unpredictable factor is the strength of local leadership in research. The record is full of instances in which the ability, foresight, or inspiration of one man led to an important turn in events. At any moment we may be blessed with another Hewitt, Criddle, Comstock, Snodgrass, or Wigglesworth; and when he comes the trend of events will alter.

Finally, the insects themselves will influence future developments in entomological research. Who, a few short years ago, could have predicted the problem of the Dutch elm disease in Quebec and Ontario, the potato-rot nematode in Prince Edward Island, the European corn borer in Manitoba and Saskatchewan, the witches'-broom of potatoes and the little cherry disease in British Columbia, the sugar beet root louse in Alberta; or the general insect rebellion against DDT that resulted in the resistant house fly and the scourge of mites; or the rapid research developments that sprung from the victory of a virus over the European spruce sawfly in New Brunswick? What does the future hold? Perhaps the answer lies as much with the grasshopper and the budworm as with the investigator.

Recapitulation

It is fully anticipated that the broad lines of today's research program will carry over to tomorrow. Sudden large-scale departures are not in prospect. However, it appears that there will be a marked and steady trend toward a more critical type of investigation conducted by teams of research specialists working in composite-type laboratories that represent segments of a closely integrated program administered through a consolidated central office. The subjects under study will still feature taxonomy, forecasting of insect outbreaks, and control through biological agents and management practices, with a rebirth of interest in insect biology and basic physiology, and a new era for insecticides. The future program must continue to be directed toward the primary objective of practical protection. In detail it will be materially affected by the course of international relations, changes in husbandry practice, advances in other fields of science, the quality of local leadership, and the insects themselves.

Pest Trichoptera at Fort Erie, Ontario[1]

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In many parts of the St. Lawrence drainage system, enormous numbers of Trichoptera, often mingled with Ephemerida, emerge during the summer and become a pest in a considerable zone bordering the water. The insects swarm in open areas, especially around trees and lights, invade houses, settle in large numbers on walls and other surfaces, ruining those that have been freshly painted, and pile in decaying and odoriferous drifts along the shore line. In addition, they give rise to a medical problem by evoking allergic reactions in hypersensitive individuals.

Along the upper reaches of the Niagara River, the incidence of Trichoptera is particularly high, and the insects have been a subject of popular comment for many years. Betten (1934) has reported the results of field work he carried out at Buffalo, N.Y., in the summer of 1906. He remarks on the abundance of Trichoptera, notes various instances of their annoying habits, and gives records of about seventeen species captured at Buffalo, as well as of several others taken at Niagara Falls. He notes that the bulk of the population at this locality is composed of Hydropsychidae, by far the most numerous species being *Hydropsyche bifida* Wlk., which he lists under the name *H. chlorotica* Hag.

Similar conditions prevail on the Canadian side of the river at Fort Erie, Ontario, and a serious interest has recently been taken in the problem at that city. As a result three collections of Trichoptera were made in the summer of 1950, so that a preliminary idea could be obtained of the composition and distribution of the swarms. Details of these collections follow.

Collection 1.—Made by Messrs. H. E. Thompson and M. E. Atrill of Fort Erie, in the period July 18-24. Adult Trichoptera, collected by hand at specified points in Fort Erie. Twenty-seven samples, comprising 1322 specimens.

Collection 2.—Made by Dr. T. N. Freeman, of the Systematic Entomology Unit, Division of Entomology, on Aug. 4 and 5. Collected by various means, in and near Fort Erie. Six samples, comprising 1081 adults and 54 larvae and pupae.

Collection 3.—Made by Messrs. D. G. Peterson, D. L. Watson, and P. E. Morrison of the Household and Medical Entomology Unit, Division of Entomology in the period Aug. 8-24. Collected by various means, in and near Fort Erie. Ninety-eight samples, comprising an estimated 8300 adults and 1400 larvae and pupae, of which 4333 adults and 1160 larvae and pupae were determined.

These collections are large enough and have been made by sufficiently varied methods to be taken as a representative sample of the more important pest species, at least as they occur in the latter part of the flight season. Betten's collections are so similar as to suggest that conditions differ little in the earlier part of the summer, although it is probable that, as in other comparable localities, a few additional species are common at that time.

The general aspect of the fauna, as represented in these collections, is one of great poverty. Only twenty-three determined species are represented in the three collections that I have studied; there are in addition possibly two undetermined species of Hydrotilidae. Betten records a further six species from Buffalo and three from Niagara Falls, bringing the total of species recorded from the region up to thirty-four.

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This small apparent number of species is no doubt partly the result of a small range of available larval habitats. All the species known from the fauna are either restricted in the larval stages to rapidly flowing water or commonly found in this environment. The complete dominance of species characteristic of rapid streams suggests that almost all the Trichoptera of the Fort Erie region breed in the Niagara River. This conclusion is supported by the fact that along the shores of Lake Erie near the Niagara River the incidence of Trichoptera is very low as compared with that along the shores of the Niagara River itself. It is also supported by the composition of the samples of larvae and pupae from the Niagara River. These indicate the presence of a very dense population of immature Trichoptera, the species or species groups being represented in approximately the same proportions as in the samples of adults. Only one species in the collections of immature forms was demonstrably not represented in the collections of adults. This was a limnephilid, of which three or four young larvae were collected.

Restriction of habitat type, however, may not in itself be a sufficient explanation of the poverty of the fauna, for at least twice as many species would normally be found in a sample of similar size from any one of several localities on the St. Lawrence or the Ottawa River, where the fauna is likewise entirely or dominantly of fluviatile origin. The total of the present collections is large numerically, but is small as compared with the enormous aggregate of individuals flying on favourable nights. This is shown by the large size of the samples that were collected, on several different occasions, in a very short space of time. Sample D of Collection 2, containing 381 specimens, chiefly of *Oecetis avara* (Banks), was collected in one minute of sweeping near a light on the Peace Bridge; and Sample A 38-41 of Collection 3, containing an estimated total of 6800 specimens, mainly of *Hydropsyche bifida*, was taken in ten sweeps at the water's edge at Old Fort Erie.

Though it is probable that additional species will be found with further collecting in the Fort Erie region, it is unlikely that their discovery will alter our concept of the fauna significantly from the economic standpoint. Of the determined adult specimens in the present collections, over 70 per cent are of a single species, *Hydropsyche bifida*; the four most abundant species make up over 90 per cent, and the seven most abundant over 95 per cent, of the same material. In the undetermined material, almost all of which comes from the single sample A 38-41 of Collection 3, a considerably higher proportion of *Hydropsyche bifida* is to be expected, as this species constituted well over 99 per cent of the determined portion of the sample. Any additional species that may be present are accordingly not likely to be numerically important in the total caddis-fly population.

In the list that follows, the species represented in the present collections are discussed in descending order of abundance.

Hydropsyche bifida Walker.—This was by far the most numerous species, outnumbering all other combined by more than two to one. The sexes were about equally represented in the determined material. Determination of all individuals in the very large sample A 37-41 of Peterson, in which females were greatly preponderant, would have raised the proportion of females considerably. In single large samples, the proportion of females varied from 2 to 93 per cent. Betten also found this species in large numbers at Buffalo, and states (1934, p. 187) that it was most abundant in June. The present collections show a decrease in abundance of the species from mid-July to early August, when several other species were more numerous. The number of individuals collected rose sharply

on August 10, and the largest number was collected on August 23. Larvae of the *bifida* group were found in numbers in the Niagara River, and no doubt most of them belonged to this species.

Oecetis avara (Banks).—606 males, 209 females. This species reached a peak of abundance on Aug. 4-5, during which period more than half of the total number of specimens were collected.

Hydropsyche placoda Betten.—104 males, 115 females. The incidence was rather uniform, small numbers of specimens appearing in most samples. This was the only species of the *scalaris* group of *Hydropsyche* that was represented in the collections of adults. Accordingly it is assumed that the larvae of this group that were collected in some numbers in the Niagara River were those of *H. placoda.*

Neureclipsis crepuscularis (Walker).—133 males, 68 females. This species appeared chiefly in collections made from swarms in the vicinity of trees and shrubs. There was no marked seasonal fluctuation of abundance. Only a few larvae were collected.

Macronemum zebratum Hagen.—138 males, 33 females. Moderate or small numbers were present in most of the samples of adults. Only one larva was collected.

Cheumatopsyche campyla Ross.—68 males, 65 females. This species appeared in small numbers in most of the samples. *Cheumatopsyche* larvae were abundant in the Niagara River.

Helicopsyche borealis Hagen.—16 males, 51 females. The representation of this species was in general scanty, but Sample 11 of Collection 1, taken on July 20, contained 3 males and 32 females. No larvae were collected.

Athripsodes cancellatus (Betten).—35 males, 25 females. There was no marked peak of abundance. Larvae of *Athripsodes* were poorly represented, although five species of the genus occurred in some numbers in the collections of adults.

Oecetis inconspicua (Walker).—13 males, 33 females. Although in most parts of Eastern Canada this is the most common species of *Oecetis*, at Fort Erie it appears to be greatly outnumbered by *O. avara.* In general, *O. inconspicua* was poorly represented in the collections, but 5 males and 28 females were taken at light on August 5, in Sample F of Collection 2.

Athripsodes tarsi-punctatus (Vorhies).—24 males, 9 females. All the specimens were taken in the period August 4 to 15.

Athripsodes mentieus (Walker).—21 males, 12 females. The species occurred sparingly up to August 15.

Athripsodes angustus (Banks).—21 males, 11 females. All the specimens were taken in the period August 4 to 15.

Athripsodes ancylus (Vorhies).—9 males, 20 females. This species was taken only in the period August 4 to 8.

Nyctiophylax vestitus (Hagen).—10 males, 1 female. The males were taken in the period August 4 to 10. The single female *Nyctiophylax,* taken on August 5, doubtless belongs to this species.

Oecetis persimilis (Banks).—2 males, 7 females. All these specimens were taken at light on August 5.

Protoptila maculata (Hagen).—2 males, 3 females. These specimens were taken at light on August 5. A pupa referable to this genus was collected in the Niagara River.

Hydropsyche recurvata Banks.—2 males, 2 females. One of the males was taken at light on August 8. The other specimens were reared from pupae collected in the Niagara River.

Leptocella exquisita (Walker).—2 males, 1 female. The specimens were collected on August 4 and 5.

Setodes incerta (Walker).—Two females collected at light on August 5 are referred to this species. The genitalia do not agree with the figure that Ross (1944) has given of those of *S. oligia*; the wing venation and other external characters agree well with those of *S. incerta* males.

Triaenodes sp.—1 male, 1 female. A male collected on July 18 is identical with that figured by Betten (1934, Pl. 39, Figs. 1-3) as *T. ignita* Walker. Betten and Mosely (1940, p. 77) showed that Walker's name applies to another species, and that the species figured by Betten was at that time nameless. This appears still to be the case; *T. baris* Ross is closely similar, but differs in the shapes of the tenth tergite and of the external ramus of the clasper. A female collected on Aug. 4 has genitalia similar to those figured by Ross for *T. baris*; it is possible, however, that this female is conspecific with the Fort Erie male, rather than with *T. baris*, which is known only from a single locality, in Illinois.

Polycentropus cinereus Hagen.—One male was taken in an emergence cage on August 23. *Polycentropus* larvae, undetermined as to species, were found in the Niagara River.

Agraylea multipunctata Curtis.—One male, without date, was taken in Collection 3. Other Hydroptilidae, belonging to possibly two additional species, appeared in very small numbers in a few of the samples.

Athripsodes submacula (Walker).—One female was collected on August 19. The species is recorded from Buffalo by Betten.

Betten has reported the following additional species from the region of the Niagara River: from Buffalo, *Dolophilus moestus* (Banks)[3], *Phylocentropus placidus* (Banks), *Leptocella albida* (Walker), *Athripsodes resurgens* (Walker), *A. dilutus* (Hagen), and *Setodes oligia* (Ross); from Niagara Falls and vicinity, *Glossosoma* sp., *Chimarra socia* Hagen, and *Hydropsyche walkeri* Betten and Mosely.

[3]The names given by Betten have been modified to accord with current ideas of synonymy.

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References

Betten, C. 1934. The caddis flies or Trichoptera of New York State. *New York State Mus. Bull.* 292.

Betten, C., and M. E. Mosely. 1940. The Francis Walker types of Trichoptera in the British Museum. London.

Ross, H. H. 1944. The caddis flies, or Trichoptera, of Illinois. *Bull. Illinois Nat. Hist. Survey* 23: 1-326.

Egg-laying Habits, Overwintering Stages, and Life-cycle of *Simulium arcticum* Mall. (Diptera: Simuliidae)[1]

By F. J. H. FREDEEN[2], J. G. REMPEL[3], AND A. P. ARNASON[4]

Investigations begun in 1947 in Saskatchewan have provided new data on the life-history and habits of the black fly *Simulium arcticum* Mall. The only published account of the biology and egg-laying habits of this species is that by Cameron (1922). He states that the eggs are laid in the usual black-fly fashion, i.e., attached in masses to rocks wetted by spray in river rapids. The evidence here reported, however, indicates that the eggs are laid singly over the surface of flowing water and that these eggs settle and become embedded in the sand on the bottom. The investigations have conclusively shown that the species overwinters in the egg stage. These facts, important in an understanding of the biology of the species, are of particular value in planning a sound program of control.

Oviposition

In his publication on the biology of *S. arcticum* Mall. [=*S. simile* Mall.], Cameron (1922) stated, "The eggs are not laid singly but in large cake-like masses, embedded in a soft, elastic, gelatinous matrix, by means of which they adhere readily to the surfaces of the stones on which they are deposited."

Investigations during 1947 and 1948 included an intensive search for eggs on rocks and vegetation in rapids, on willows on the beach, and in sand under these willows. None was found and it was concluded that they were being laid elsewhere.

Throughout June, July, and August gravid females were collected from vegetation on the river flats along the Saskatchewan River. Observations and sweep-net collections, throughout several 24-hour periods, indicated that the black flies had a definite cycle of activity. During the heat of the day they clung motionless to the undersurfaces of willow leaves and to the shaded portions of boulders. None was observed over the water. At sundown, when the air became cooler and calmer, the black flies took wing, only the occasional individual being observed to return, and that briefly. Observations were then made from a boat on the river, and it was found that during calm, warm evenings the black flies swarmed low over the water surface, and some were observed touching the water. Sweep-net collections from these swarms yielded only gravid females of *S. arcticum.*

When gravid females were swarming over the water surface, collections of the surface film were made with a Wisconsin-type plankton net towed behind a motor boat. Two collections, each representing about 5,000 square feet of surface film, yielded a total of 15 black-fly eggs. These eggs varied in colour from a pale cream to dark brown. They were undeveloped embryologically and did not hatch when placed in water at 70°F.

Some of the gravid flies, identified as *S. arcticum,* were collected with a sweep net from swarms over the river surface and were placed in small cages. Egg masses were subsequently found attached to the glass walls of one of the cages. The eggs in these masses lacked the protective, sticky matrix in which the eggs of other species of *Simulium* are embedded. They were kept in water at room temperature in the laboratory for two months, during which time no

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apparent embryological development occurred. Several single black-fly eggs were found on the bottom of a second cage.

The failure to find eggs or egg masses on rocks or other objects in the river, together with the discovery of individual eggs floating in the river, suggested that they probably settled to the bottom and became embedded in the silt and sand of the river bed. Accordingly, dredgings and other collections were made from the river bottom at various places. These have conclusively demonstrated that *S. arcticum* eggs are distributed over the river bed.

Distribution of Eggs

An Ekman dredge was used to collect quantitative samples of sand from the river bed. Twenty-two series of dredgings were taken from the Saskatchewan River in more than two and one-half years. In addition, 16 series of samples were taken from sand above the water line on the river shore. The number of eggs in the samples was small, never being greater than one in three cubic inches of sand. Therefore, large samples, each consisting of 15 to 30 dredgings, were required. The eggs from these were extracted by a salt flotation process during which the samples were stirred gradually into a saturated solution of NaCl at room temperature. This caused the organic material, including black-fly eggs, to float so that it could be skimmed off and examined under a microscope. The eggs were placed in tap water, counted, and their hatchability determined. The flotation treatment did not affect the viability of the eggs unless they were exposed to salt solution for several hours and became plasmolysed.

During the summer of 1948 five series of dredgings were taken from the south branch of the Saskatchewan River, and two from the north branch. Fewer than three eggs per square foot of bottom sand were found in the south branch, and one egg per square foot in the north branch at Prince Albert. No eggs were recovered from samples taken 95 miles upstream from Prince Albert.

Samples of bottom sand, one inch thick, were collected through the ice of the South Saskatchewan River at Saskatoon during the winter of 1948-49. These contained 24 to 46 eggs per square foot. Seven per cent of the eggs collected in December and 95 per cent of those collected in March hatched.

Less than one egg per square foot and numerous egg shells were found in the same Saskatoon area during the last two weeks of May, 1949, after first-instar larvae had appeared in the river. Two to six eggs per square foot were found in this area during the egg-laying season of July, 1949, and fewer than two per square foot in October as the river was beginning to freeze over. The eggs collected in July and October failed to hatch in the laboratory.

Eleven eggs per square foot were found in sand collected through the river ice at Saskatoon in March, 1950. Thirty-four per cent of these eggs hatched. The following October, three eggs per square foot were found and two per cent of these hatched.

Black-fly eggs were also found in sand collected in the spring from river flats that had been flooded during the oviposition period in June and July, but none was found at higher shore levels. These eggs, including some that were found in the early spring in sand that had been one and one-half feet above the river ice during the winter, hatched when placed in water in the laboratory.

Viable black-fly eggs were found as deep as four inches in sand on the river bottom, no doubt buried by the shifting sand. On May 16 and 23, 1949, immediately after the spring hatch, the shells of black-fly eggs were more abundant in the uppermost three inches of sand in the river bed than were unhatched eggs, but at the depth of three to four inches eggs were nine times as abundant as egg

shells. Two months earlier, prior to egg-hatching, eggs were abundant in the surface layer of sand in the river bed and no shells were found. These findings indicate that eggs hatch in the sand at a depth as great as three inches and that larvae are able to reach the surface.

Overwintering Stages

This species overwinters mainly in the egg stage. Eggs were found in the river bed in all seasons of the year but were most abundant in the winter. On one occasion small numbers of larvae were found in the winter and the spring. Pupae were never encountered between late fall and early spring. Great numbers of newly hatched larvae have been generally found soon after the ice break-up in the spring. Moreover, eggs deposited at times of high water level during June and July survived the winter in sand well above the ice level.

Even though oviposition occurs mainly in June and July, eggs are apparently not ready to hatch until the following spring. No more than seven per cent of the eggs collected in the summer, fall, and early winter months hatched in the laboratory, but a large percentage of eggs collected in March hatched almost immediately when placed in water. A very slow rate of embryological development (or some physiological block) that prevents hatching until the spring after oviposition is therefore indicated.

In appraising control experiments it was observed that in parts of the river where larvae had been eliminated in May and June, first-instar larvae appeared in considerable numbers within a few days. As gravid females were not present at this time, these larvae must have hatched from eggs laid the previous year. Further observations in these sections of the river revealed that small numbers of first-instar larvae continued to appear, sometimes as late as mid-September, all presumably from eggs deposited the previous year.

Life-cycle

These studies indicate that *S. arcticum* has one generation only each year in the central part of Saskatchewan and that in this region the species overwinters mainly as eggs sparsely distributed in the sand of the Saskatchewan River bed. The eggs commence hatching in the spring one to two weeks after the ice breaks up and when the water has reached an average daily temperature at 47° to 49°F.; the majority hatch at that time, but small numbers of first-instar larvae continue to appear throughout most of the ice-free season. First-instar larvae, widely distributed over the bottom, move downstream with the current. They first attach in large numbers to submerged willows and to small stones and gravel on flooded beaches, but disappear from these sites; the later instars are found massed chiefly on boulders in swift rapids. Larvae develop rapidly, and pupation begins about four weeks after the first appearance of larvae and reaches a peak about a week later. Mass emergence of adults during the latter part of May or in June sometimes results in extensive losses of livestock (Rempel and Arnason, 1947). Oviposition occurs chiefly in late June or July. The eggs are laid singly as the black flies swarm over the surface of the river; and these eggs settle to the bottom, where some become buried under several inches of sand.

The long period of hatching and the movement of larvae in the river both have an important bearing on black-fly control. For example, a considerable section of the Saskatchewan River that was cleared of black-fly larvae by the application of a DDT larvicide (Arnason *et al.*, 1949) on May 25, 1948, was reinfested within two weeks by early-instar larvae. The reinfestations were heaviest in those rapids near the upstream end of the cleared area, indicating a movement of larvae from egg beds and untreated rapids upstream. In 1949 and 1950 the amount of reinfestation after control tests in May and in late June was

sufficient to warrant repeated control applications. These reinfestations were caused by first-instar larvae that originated from eggs that did not hatch until after the treatments were made. As a result of this long hatching period, repeated applications of larvicide in one season may be required to effect satisfactory control.

Summary

Studies conducted along the Saskatchewan River from 1947 to 1950 indicate that *Simulium arcticum* Mall. oviposits generally over the surface of the river water during July. The eggs are laid singly, sink to the bottom, and may become embedded several inches in the sand. The species overwinters almost entirely in the egg stage. The eggs begin to hatch in the spring soon after the ice clears from the river, and the larvae move downstream and attach themselves to rocks in rapids to complete their development. The control of this black fly may require repeated applications of larvicide because of a long hatching period.

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References

Arnason, A. P., A. W. A. Brown, F. J. H. Fredeen, W. W. Hopewell, and J. G. Rempel. Experiments in the control of *Simulium arcticum* Malloch by means of DDT in the Saskatchewan River. *Sci. Agr. 29:* 527-537. 1949.

Cameron, A. E. The morphology and biology of a Canadian cattle-infesting black fly, *Simulium simile* Mall. (Diptera: Simuliidae). Canada Dept. Agr., (Tech.) Bull. 5 (n.s.). 1922.

Rempel, J. G., and A. P. Arnason. An account of three successive outbreaks of the black fly, *Simulium arcticum*, a serious livestock pest in Saskatchewan. *Sci. Agr. 27:* 428-445. 1947.

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